

Claims.

1. A method of measuring the production of a target analyte of interest in a human or animal, comprising the steps of:

- a. injecting a human or animal with an appropriate amount of targeting moiety capable of binding specifically to the target analyte;
- b. allowing the targeting moiety to circulate through the injected human or animal for a time sufficient to bind to the target analyte of interest and form a targeting moiety:target analyte conjugate;
- c. obtaining a sample of body fluid from the human or animal without dissociation of the target analyte from targeting moiety;
- d. combining the sample of body fluid with a capture moiety capable of binding specifically to the analyte determinants of the targeting moiety:target analyte conjugate;
- e. incubating the assay mixture to allow the immobilized capture moiety to bind specifically to either the target analyte or the labeled targeting moiety;
- f. removing unbound targeting moiety and target analyte from the capture moiety;
- g. detecting the bound conjugate on the capture moiety; and
- h. determining the amount of the target analyte in the sample.

2. The method of claim 1, wherein the target analyte is a macromolecule.

3. The method of claim 2, wherein the macromolecule is a protein.

4. The method of claim 3, wherein the protein is a cytokine

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Sub
D2

- Sub 517
5. The method of claim 4, wherein the cytokine is selected from the group consisting of interleukins, interferons chemokines, growth factors, colony stimulating factors, lymphokines, lymphotoxins, and tumor necrosis factors.
6. The method of claim 4, wherein the cytokine is selected from the group consisting of interleukin-1, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, interleukin-17, interleukin-18, , interferon-alpha, interferon-beta, interferon-gamma, lymphotoxin, tumor necrosis factor-alpha, TGF-beta, GM-CSF, NGF, and EGF.
- Sub C2
- 10 7. The method of claim 1, wherein the body fluid is selected from the group consisting of saliva, blood and extracellular fluid.
8. The method of claim 1, wherein the targeting moiety is selected from the group consisting of antibodies, soluble receptors, paratopic molecules, recombinant molecules with binding sites for the target analyte, and fragments thereof.
- Sub a2
- 15 9. The method of claim 8, wherein the targeting moiety is an antibody.
10. The method of claim 1, wherein the capture moiety is an antibody.
11. The method of claim 10, wherein the ^{capture}antibody is a polyclonal antibody which recognizes many epitopes on the target analyte.
12. The method of claim 1, wherein the targeting moiety is detectably labeled through the use of a label selected from the group consisting of radioisotopes, affinity labels, enzymatic labels, and fluorescent labels.
- Sub a3

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Sub D4
13. The method of claim 1, wherein the targeting moiety is labeled by linking the targeting moiety to a label which label can then be bound to a binding partner which is conjugated to an enzyme.

Sub D5
14. The method of claim 13, wherein the label is a small molecule hapten.

5 15. The method of claim 14, wherein the hapten is biotin.

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16. The method of claim 13, wherein the enzyme-conjugated binding partner is selected from the group consisting of streptavidin, anti-biotin antibody, anti-hapten antibody, and anti-immunoglobulin antibody.

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10 17. The method of claim 13, wherein the enzyme is selected from the group consisting of alkaline phosphatase, glucose oxidase, beta-galactosidase, malate dehydrogenase, staphylococcal nuclease, delta-5 steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

15 18. The method of claim 12, wherein the targeting moiety is labeled by linking to a fluorescent labeling compound.

19. The method of claim 18, wherein the fluorescent labeling compound is selected from the group consisting of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

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20 20. The method of claim 8, wherein the targeting moiety is itself capable of being bound by another molecule. a

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21. The method of claim 20, wherein the targeting moiety is an antibody.

22. The method of claim 21, wherein the molecule capable of binding the targeting moiety is an antibody which recognizes the targeting moiety.
23. The method of claim 22, wherein the antibody which recognizes the targeting moiety is a polyclonal antibody which recognizes many epitopes on the targeting moiety.
- 5 24. The method of claim 20, wherein the capture moiety is an antibody. ?
25. The method of claim 20, wherein the means for detecting the bound conjugate on the solid support is by radioimmunoassay, wherein the molecule capable of binding the targeting moiety is labeled by linking the targeting moiety to a radioisotope.
26. The method of claim 20, wherein the molecule capable of binding the targeting moiety is detectably labeled by linking it to a label which label can then be bound to a binding partner which is conjugated to an enzyme.
27. The method of claim 26, wherein the label is a small molecule hapten.
28. The method of claim 27, wherein the hapten is biotin.
29. The method of claim 26, wherein the enzyme-conjugated binding partner is selected
- 15 from the group consisting of streptavidin, anti- biotin antibody, anti-hapten antibody, and anti-immunoglobulin antibody.
30. The method of claim 26, wherein the enzyme is selected from the group consisting of alkaline phosphatase, glucose oxidase, beta -galactosidase, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase,
- 20 alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, asparaginase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

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2nd targeting moiety

Sub D9
31. The method of claim 20, wherein the molecule capable of binding the targeting moiety is labeled by linking to a fluorescent labeling compound.

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32. The method of claim 31, wherein the fluorescent labeling compound is selected from the group consisting of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

5 33. The method of claim 1, wherein the capture moiety is immobilized on a solid phase support.

34. A reagent kit useful in performing the method of claim 1, comprising

- 10 (a) a first reagent containing a labeled targeting moiety specific for the target analyte and capable of forming a conjugate with the target analyte;
- (b) a second reagent separated from said first reagent which contains a capture moiety for said conjugate; and
- (c) a third reagent separated from said first and second reagents which contains a standard for the analyte.

15 35. The reagent kit of claim 34, wherein the targeting moiety is an antibody.

36. The reagent kit of claim 34, wherein the capture moiety is an antibody.

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37. A reagent kit useful in performing the method of claim 1, comprising: (a) a first container having paratopic molecules that immunoreact with a target analyte, and are operatively linked to an enzyme indicating means; (b) a second container having paratopic molecules that immunoreact with the target analyte at a site different from the first paratopic molecules but are not in the first container; and (c) one or more other containers comprising one or more of the following: a sample reservoir, a solid phase support, wash reagents, reagents capable of detecting presence of bound

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antibody from the second container, or reagents capable of amplifying the indication means.

38. A reagent kit of claim 37, wherein the paratopic molecules are detectably labeled through the use of a label selected from the group consisting of radioisotopes, affinity labels, enzymatic labels, and fluorescent labels.

39. A reagent kit of claim 37, wherein the paratopic molecules are detectably labeled through the use of fluorescent labeling agents are fluorochromes selected from the group consisting of fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 and sulphonyl chloride (RB 200 SC).

40. A reagent kit of claim 35, wherein the antibodies are polyclonal.

41. A reagent kit of claim 35, wherein the antibodies are monoclonal.

42. A reagent kit of claim 36, wherein the antibodies are immobilized on a solid support.